

Supplementary Information (SI)

Surface-Immobilized and Self-Shaped DNA Hydrogel and its Application for Biosensing

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Experimental

Materials

All oligonucleotides used in this research (Table S1) and dNTPs were supplied by TaKaRa Biotechnology Co., Ltd. (Dalian, China). Hydrogen peroxide (H_2O_2), acetone, and glutaraldehyde were purchased from Sinopharm Chemical Reagent Co. Ltd. Phi29 DNA polymerase, Exonuclease III and Bsr DI were from New England Biolabs Inc. bilirubin oxidase (BOD), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Horseradish Peroxidase (HRP), bovine serum albumin (BSA), and bilirubin were purchased from Sigma-Aldrich. Bilirubin measurement kit was supplied by BaiDing biological engineering (Beijing) co., LTD. HL60 cell was from Shanghai GeneChem Co., Ltd. DAPI and Cy3 were purchased from LITTLE-PA Sciences Co. Ltd. Goat anti-rabbit IgG secondary antibody-Cy3 (Cy3-IgG) was from Thermo Fisher Scientific Inc. All the reagents were of analytical-reagent grade. Water used throughout all experiments was purified by a Milli-Q system (Branstead, USA) to a specific resistance of $> 18 \text{ M}\Omega\cdot\text{cm}$.

A thermostats cultivating shaker (Eppendorf, Germany) was used to provide a certain temperature and rotation speed in the experiment. Atomic force microscopy (AFM) experiments were conducted using a commercial AFM (Agilent 5500 AFM system, Agilent Technologies, USA). UV-Vis absorption spectra were recorded by an UV-2450 UV/Vis Spectrophotometer (Shimadzu, JPN). Electrochemical measurements were performed on a model 660C Electrochemical Analyzer (CHI Instruments). Confocal images were obtained using a Leica LSM710 confocal laser scanning microscope (Leica Microsystems, Occult International Ltd, Germany).

All experiments were performed in compliance with the relevant laws and guidelines, and were approved by Ethics Committee of School of Life Sciences of Shanghai University. Informed consent was obtained for any

experimentation with human subjects.

Fabrication of surface-immobilized DNA hydrogel

To achieve the formation of DNA hydrogel at indium-tin oxide (ITO) surface, the ITO electrode was first ultrasonicated in acetone, ethanol and ultrapure water for 20 min, respectively. Then, the electrode was activated by 0.5 M ethanol (containing 5% APTES) and incubated overnight at 4 °C. After that, the electrode was cleaned with ethanol three times, followed by heating in water at 80 °C for 10 min.¹

To modify the electrode with the Primer I, the ITO electrode was first heating in glutaraldehyde at 37 °C for 2 h. Then electrode was washed with water and reaction buffer (50 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 10 mM MgCl₂, 200 µg/ml BSA, 50 mM dNTPs), then dried with nitrogen. Finally, the preparation of surface-immobilized DNA hydrogel was started using a reported protocol with some modification.² The procedure in details as follows, the electrode was first immersed in reaction buffer (containing 20 µM Primer I) for 2 h at 37 °C. After self-assembly of Primer I, 5 µM circle DNA and 1 unit/µl Phi 29 polymerase were then added to initiate LRCA (linear rolling circle amplification) reaction and incubated at 30 °C for 4 h. Subsequently, 5 µM Primer II and 5 µM Primer III were added to launch LRCA and reacted at 30 °C for 20 h. Finally, the solution was heated at 65 °C for 10 min to terminate the reaction. After cooling down to 25 °C, as a result, surface-immobilized DNA hydrogel precursor was fabricated on ITO electrode interface.

Agarose gel electrophoresis assay of DNA amplification product

DNA amplification was allowed to react in aqueous solution. 5 µM Primer I, 5 µM circle DNA and 1 unit/µl Phi 29 polymerase were mixed in the reaction buffer to initiate LRCA, and then incubated at 30 °C for 4 h. 5 µM Primer II and 5 µM Primer III were further added to launch MRCA by reacting at 30 °C for 20 h. The

freshly prepared 2% agarose gel was used to perform the agarose gel electrophoresis analysis. 5 μL of the above DNA amplification sample together with 1 μL 6 \times loading buffer containing fluorescent dye GelRed was loaded onto the well. The electrophoresis was carried out at a constant voltage of 100 V for 25 min.

Fabrication of surface-immobilized enzyme@DNA hydrogel

Schematic preparation process of surface-immobilized HRP@DNA hydrogel is depicted in Scheme 1A. In details, the mixture of 100 μL surface-immobilized DNA hydrogel precursor was incubated at 90 $^{\circ}\text{C}$ for 15 min and then cooled down to 40 $^{\circ}\text{C}$. After that, HRP with a final concentration of 911 μM was added, followed by incubating at 4 $^{\circ}\text{C}$ for 30 min on a rotary shaker at 200 rpm. Finally, after being centrifuged at 6000 rpm at 4 $^{\circ}\text{C}$ for 10 min and washed twice, the HRP@DNA hydrogel was fabricated at ITO electrode surface.

According to the process of fabrication of surface-immobilized HRP@DNA hydrogel, 0.02 U of BOD were encapsulated in 100 μL DNA hydrogel and thus formed surface-immobilized BOD@DNA hydrogel.

Detection of H_2O_2 using surface-immobilized HRP@DNA hydrogel

Colorimetric measurement: 0.2 mM ABTS and different concentrations of H_2O_2 working as the substrates were incubated with surface-immobilized HRP@DNA hydrogel in 10 mM PBS buffer (pH = 7.0). After catalysis at 25 $^{\circ}\text{C}$ for 15 min, the absorbance of the solution was recorded at a wavelength of 414 nm using a microplate spectrophotometer.

Electrochemical measurement: A three-electrode system was used for the measurement, in which the integrated DNA hydrogel ITO electrode was adopted as the working electrode. A saturated calomel reference electrode and a platinum electrode served as the reference and counter electrode, respectively. The parameters for electrochemical measurements have been

described in the captions of Figures without repetitious description here.^{3,4}

Detection of bilirubin using surface-immobilized BOD@DNA hydrogel

The surface-immobilized BOD@DNA hydrogels were mixed with different concentrations of bilirubin, and incubated in 0.1 M Tris-HCl buffer (pH = 8.1) at 37 °C for 30 min, respectively. The absorbance of the solution was recorded at a wavelength of 440 nm. Serum samples from patients who suffered jaundice were provided by Shanghai Pulmonary Hospital. Serum samples were collected from twenty jaundice patients and healthy individuals. Serum bilirubin was detected by using conventional vanadic acid oxidation method and surface-immobilized BOD@DNA hydrogels, respectively. In vanadic acid oxidation method, 50 μ L volumes of dilutions of serum were added to the bilirubin measurement kit. For the latter, 50 μ L volumes of dilutions of serum were mixed with surface-immobilized BOD@DNA hydrogels in 0.1 M Tris-HCl buffer (pH = 8.1). The serum bilirubin assays were carried out at 37 °C to start the BOD reaction. After 30min, the decrease in absorbance at 440 nm was recorded.

Fluorescence microscopy imaging

The surface-immobilized DNA hydrogel was first stained with SYBR green I (10 \times), followed by washing with water. After that, 20 μ M Cy3, 20 μ M Cy3-DNA, 20 μ M Cy3-IgG or 1 \times 10⁵ cells/ml HL60 cells (stained with DAPI) were then incubated with DNA hydrogel, respectively. Images were obtained from the same region at different time points. Images were captured at 10 \times magnification in three fluorescent channels: green channel (fluorescent signal of SYBR Green I) with excitation of 488 nm and emission of 505~550 nm, red channel (fluorescent signal of Cy3) with excitation of 546 nm and emission of 575~640 nm and blue channel (fluorescent signal of DAPI) with excitation of 340 nm and emission of 400~650 nm.

Atomic force microscopic measurements

AFM measurement was conducted using soft silicon AFM probes with contact mode and normal resonance frequency of 68 kHz. In a $(\sim 10\ \mu\text{m})^2$ region, an 8×8 grid of indentations was acquired with $2\ \mu\text{ms}^{-1}$ approach and retract speeds with a trigger force of 1 nN. The Young's modulus was calculated by AFM in Hertz model.⁵

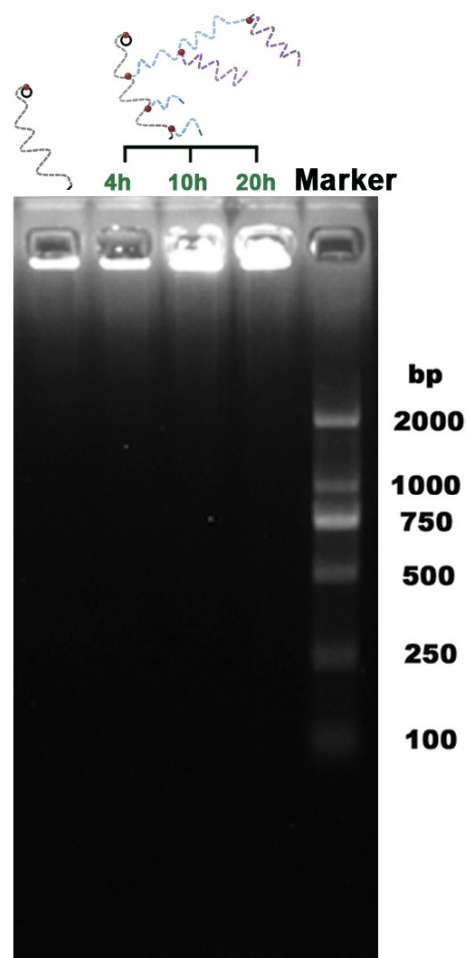


Fig. S1 Agarose gel electrophoresis assay of DNA amplification products. Lanes from left to right: LRCA product; MRCA products under different reaction time of 4 h, 10 h, and 20 h, respectively; DNA marker.

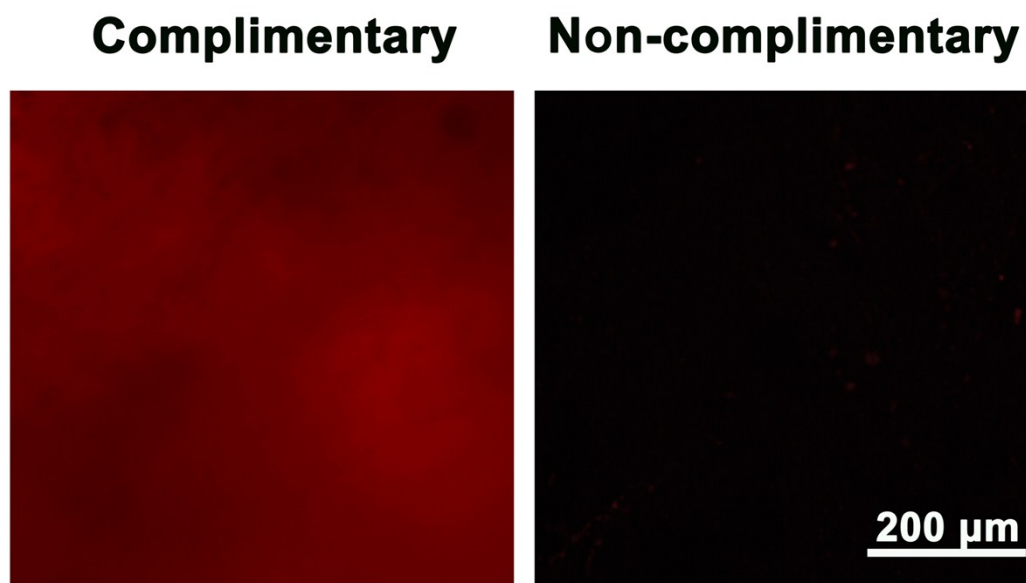


Fig. S2 Confocal fluorescence images of LRCA product on ITO electrode. Left image: A (Cy3)-labeled DNA probe, complimentary to the LRCA-produced long linear ssDNA, was adopted. Right image: A Cy3-labeled non-complimentary DNA probe was adopted as control. The sequences of the probes are shown in Table S1.

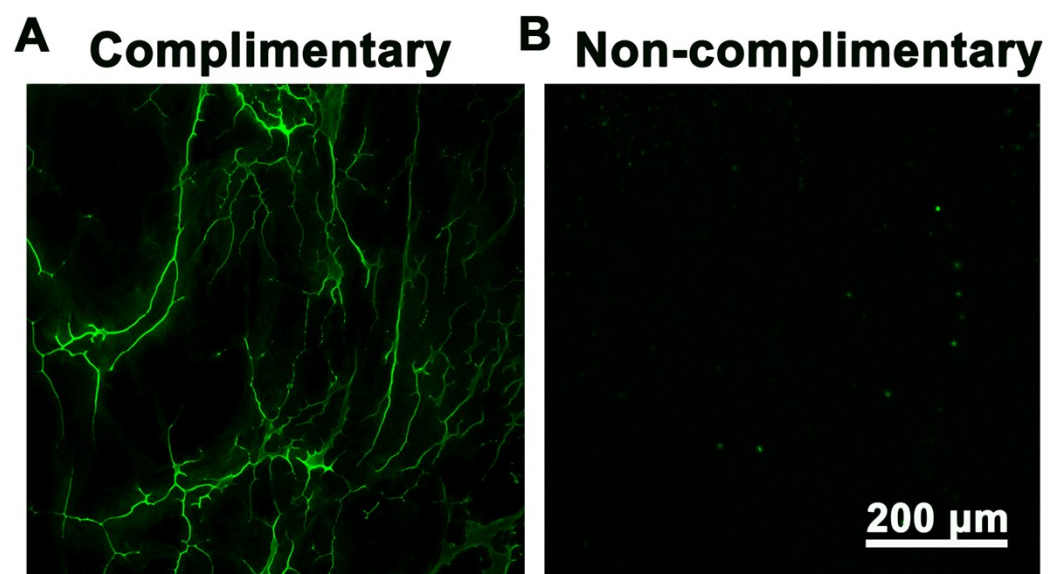


Fig. S3 Confocal fluorescence images of MRCA product on ITO electrode. The product was stained with SYBR green I (10×). The MRCA product was obtained by using (A) Primer II and Primer III, and (B) random non-complimentary Primer II and Primer III as control. The sequences of the primers are shown in Table S1.

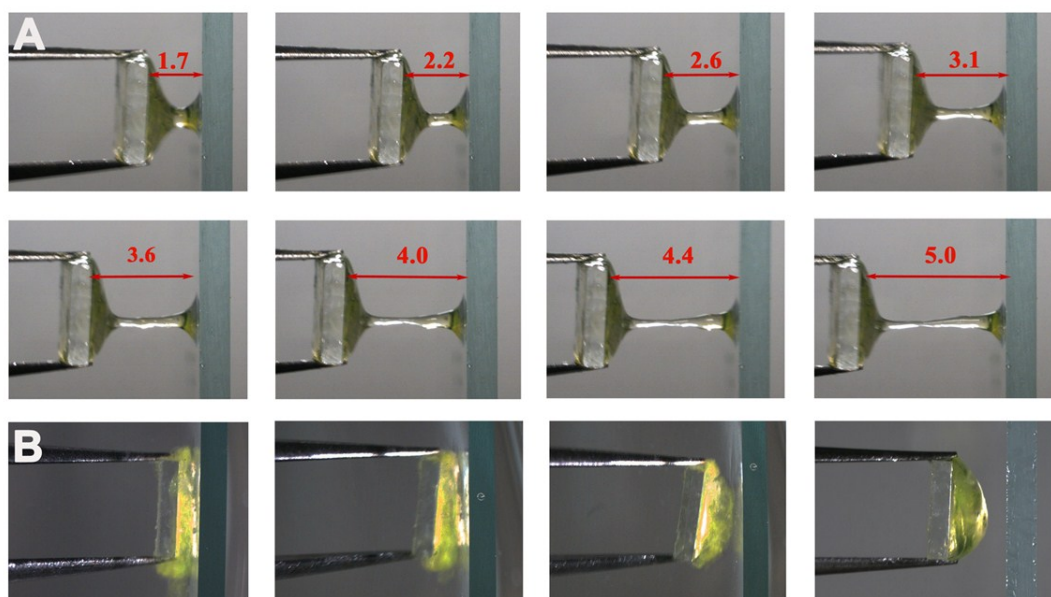


Fig. S4 The surface-immobilized DNA hydrogel was glued to glass slide in (A) air or (B) in water, and then stretched. The DNA hydrogel was stained with SYBR green I (10 \times).

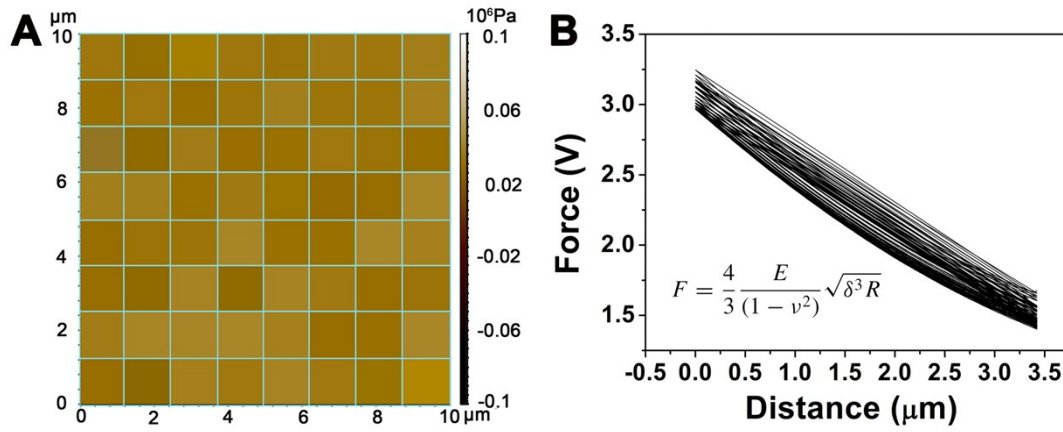


Fig. S5 (A) AFM image of the Young's modulus of the surface-immobilized DNA hydrogel in Hertz model. 64 dots were picked up in an area of 10 μm×10 μm. (B) The representative approach and retraction curves (black lines) were measured on surface-immobilized DNA hydrogel. Cantilever stiffness = 1 nN, approach and retract velocity = 2 μms⁻¹. Insert: The computational formula of Young's modulus.

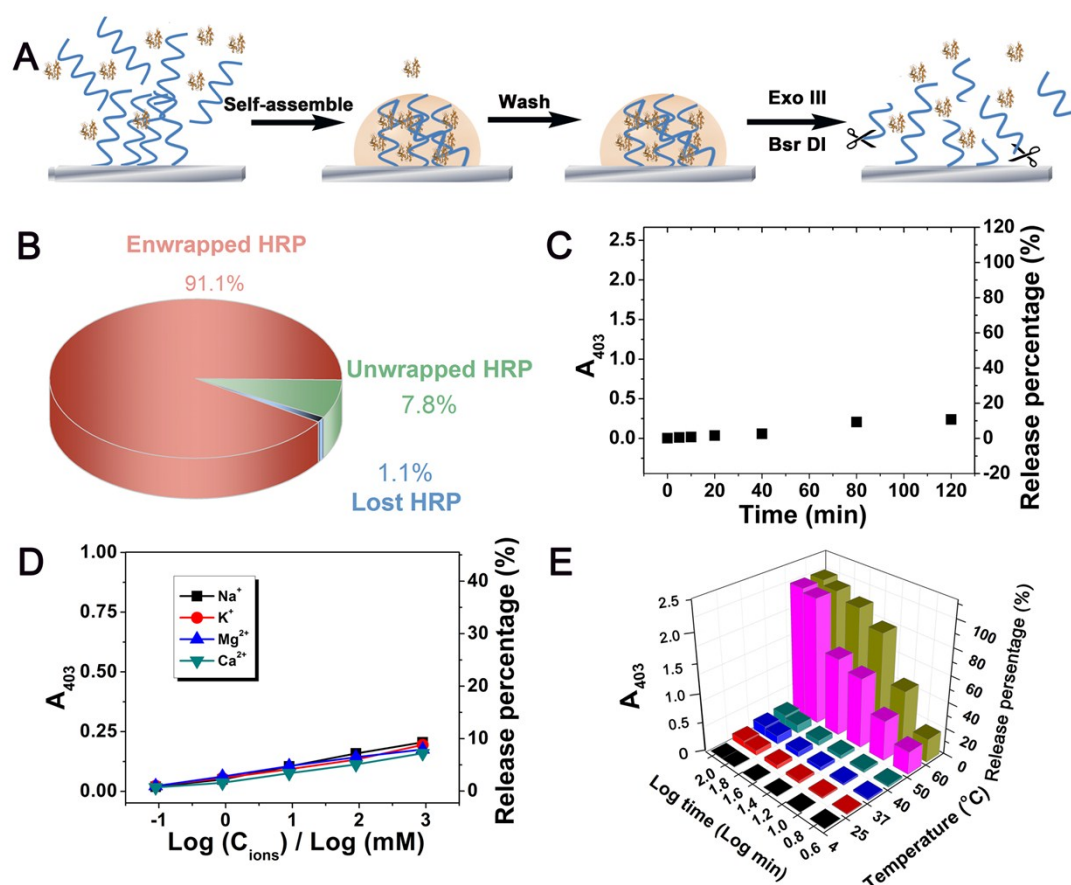


Fig. S6 (A) Schematic illustration of the process of encapsulated and released HRP of DNA hydrogel. (B) The loading capacity of surface-immobilized DNA hydrogel. The stability of surface-immobilized HRP@DNA hydrogel under different conditions at 25 °C for 2 h, including (C) buffer solution (10 mM PBS buffer, pH = 7.0), (D) metal ions and (E) temperature. Metal ions: The prepared surface-immobilized HRP@DNA hydrogel was mixed with different concentrations (0, 1, 10, 100, and 100 mM) of Na^+ , K^+ , Mg^{2+} , and Ca^{2+} , respectively.

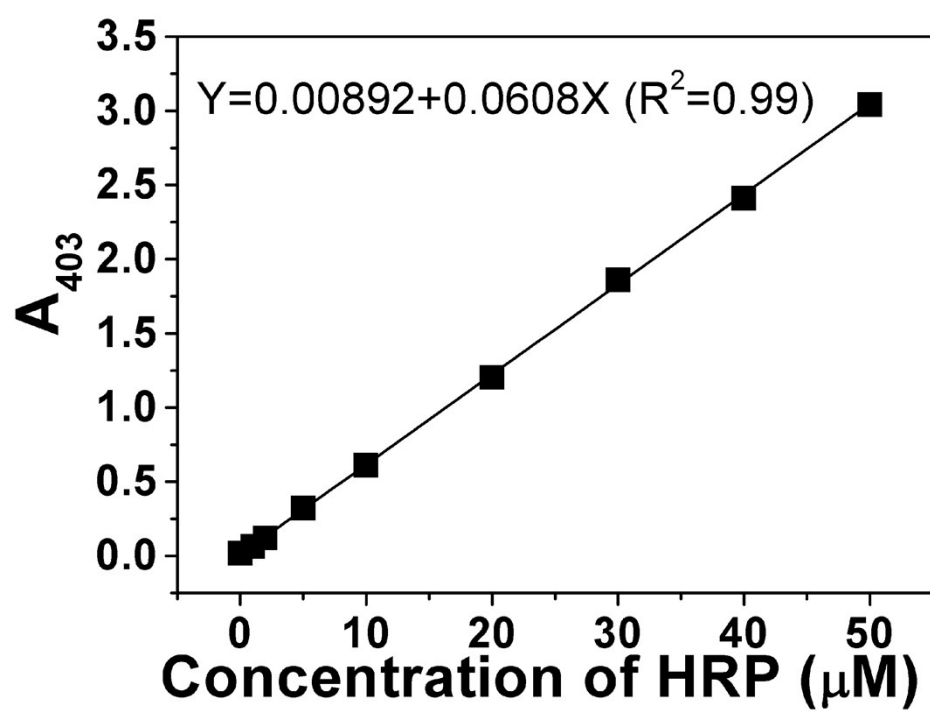


Fig. S7 The standard curve of HRP ranging from 0~50 μM. The absorbance at 403 nm of HRP was recorded.

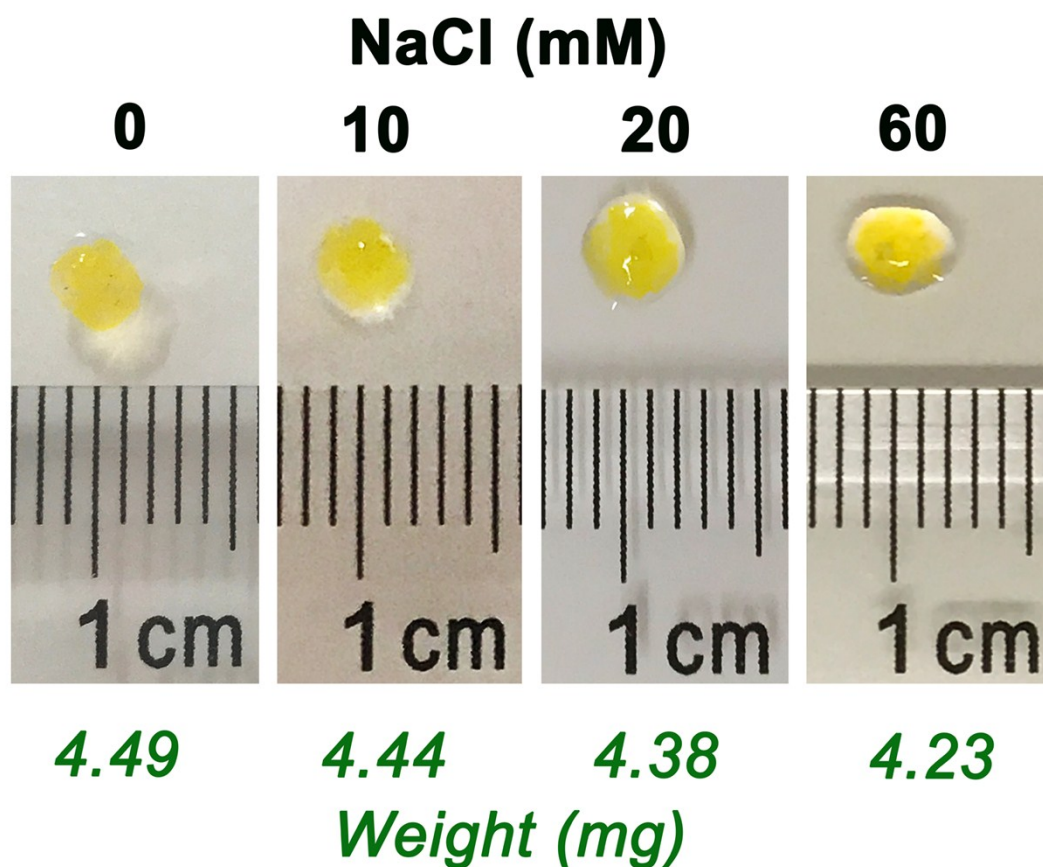


Fig. S8 Photographs of the swelling behaviour of DNA hydrogel in a solution with different concentrations of NaCl. The hydrogel was first soaked overnight in H₂O, followed by staining with SYBR green I (10×) to make it visible. Finally, the hydrogel was immersed in a solution with different concentrations of NaCl for 1 h, and was ready for photographing.⁶

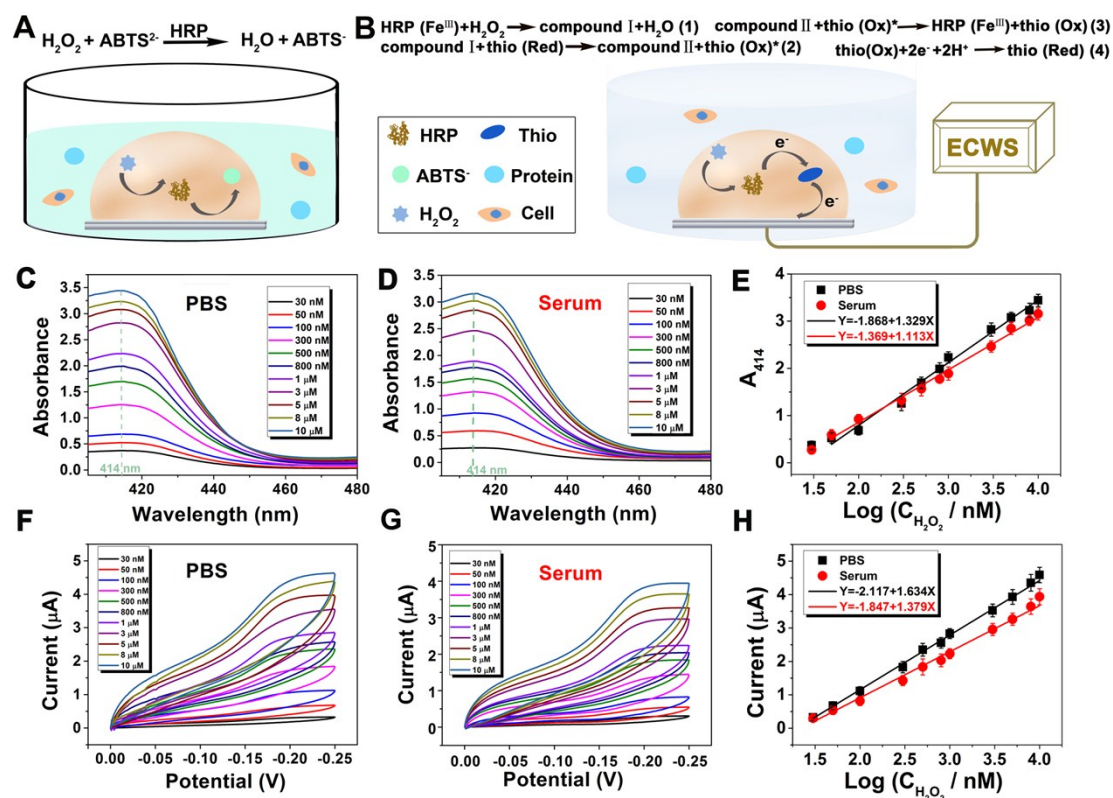


Fig. S9 (A) Schematic representation of colourimetric detection of H_2O_2 . (B) Schematic illustration of electrochemical analysis of H_2O_2 and the equations of electrochemical catalytic process. Where “Ox” denotes the oxidative states and “Red” indicates the reductive states. The electrochemical workstation (ECWS) provided two electrons in equation (4), representing the catalytic wave. Colorimetric measurements of H_2O_2 in (C) PBS or (D) serum using the catalysis of surface-immobilized HRP@DNA hydrogel in 10 mM PBS buffer (pH = 7.0) at 25 °C for 15 min. (E) The corresponding plot of UV-vis absorption at 414 nm of the product ABTS^\bullet against the concentration of H_2O_2 . The concentration of ABTS was 0.2 mM. Cyclic voltammograms obtained at HRP@DNA hydrogel modified ITO electrode in (F) PBS or (G) serum. (H) The relationship between the net peak current at -0.25 V (blank response has been subtracted from each data point) and the concentration of H_2O_2 . Test solution: 10 mM PBS containing 20 M thionine and 6 mM H_2O_2 . Scan rate was 5 mV s^{-1} .

Table S1. Oligonucleotides sequences used to prepare surface-immobilized DNA hydrogel.

DNA	Sequence (5'-3')
Circular DNA	AAA ATC TCT AGC AGT CCC ACC CTC CAA CCA CCA AGG CAA TGT ACA CGA ATT CGC CGA ACG CAC GCG ATC CGC ATG TGG
Primer I	ACT GCT AGA GAT TTT CCA CAT
Primer II	ACC AAG GCA ATG TAC ACG AAT TC
Primer III	CAT GCG GAT CGC GTG CGT
non-complimentary Primer II (control)	TCT ACA CTG ACA GCG TA
non-complimentary Primer III (control)	CCG TAA CTT GAC TGT C
Cy3-labeled probe	Cy3-ACG CAC GCG ATC CGC
Cy3-labeled non- complimentary probe	Cy3-TAGGAGTATATCGTCTGCT ACTGT

Table S2. Detection of bilirubin in real serum samples using vanadic acid oxidation method (VAO) and surface-immobilized BOD@DNA hydrogel, respectively. The concentrations of bilirubin are calculated from the standard curves (referred to Fig. 3A).

No.	Group	VAO ^a			C _{BOD@DNA hydrogel} ^b			Relative deviation ^c (%)
		ΔA_{440} ^d	μM	mg/L	ΔA_{440}	μM	mg/L	
1	Patient	0.9870	159.17	93.06	0.6693	164.58	96.22	3.4
2		2.3925	393.42	230.02	1.5945	395.87	231.45	0.6
3		0.5432	85.20	49.81	0.3578	86.70	50.69	1.8
4		0.3755	57.25	33.47	0.2467	58.92	34.45	2.9
5		0.1600	21.33	12.47	0.0926	20.41	11.93	-4.3
6		0.8214	131.56	76.92	0.5420	132.76	77.62	0.9
7		0.2670	39.17	22.90	0.1735	40.63	23.75	3.7
8		1.1599	187.98	109.90	0.7686	189.40	110.73	0.8
9		0.7082	112.70	65.89	0.4748	115.94	67.79	2.9
10		2.1671	355.85	208.05	1.4278	354.21	207.09	-0.5
11	Healthy	0.1130	13.50	7.89	0.0690	14.51	8.48	7.5
12		0.0762	7.37	4.31	0.0455	7.83	4.58	6.2
13		0.0456	2.26	1.32	0.0178	2.01	1.18	-11.1
14		0.0537	3.61	2.11	0.0247	3.42	2.00	-5.3
15		0.0837	8.62	5.04	0.0504	9.46	5.53	9.7
16		0.0586	4.43	2.59	0.0322	4.81	2.81	8.5
17		0.1004	11.40	6.67	0.0611	12.52	7.32	9.8
18		0.0421	1.69	0.99	0.0162	1.49	0.87	-11.8
19		0.0819	8.32	4.86	0.0494	9.60	5.61	15.4
20		0.1227	15.11	8.83	0.0752	16.05	9.38	6.2

^aThe concentrations of bilirubin in real serum samples detected by VAO; ^bThe concentrations of bilirubin in real serum samples detected by BOD@DNA hydrogel;

^cThe relative deviation is calculated using an equation as follows: $(RD = (C_{\text{BOD@DNA hydrogel}} - C_{\text{VAO}}) / C_{\text{VAO}})$ (RD: relative deviation, C_{VAO} : concentration obtained using VAO, $C_{\text{BOD@DNA hydrogel}}$: Concentration obtained using $C_{\text{BOD@DNA hydrogel}}$); ^d ΔA_{440} are the absolute decreased values of the absorbance of the substrate bilirubin at 440 nm.

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